Aus dem Experimental and Clinical Research Center
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DISSERTATION

MACC1 regulates death receptor mediated apoptosis in solid cancers through STAT1/3 – Mcl-1 signaling

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von

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.....dedicated to my parents, teachers and friends

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Zusammenfassung

Einleitung: Apoptose ist ein grundlegender zellulärer Mechanismus, der in der Krebsentstehung oft dereguliert ist. MACC1 (Metastasis associated in colon cancer 1) wurde vor kurzem als eines der Schlüsselfaktoren der Tumorprogression und –metastasierung identifiziert. Das Ziel dieser Arbeit ist, den Einfluss von MACC1 auf die Regulation der extrinsischen, durch Todesrezeptoren vermittelten Apoptose in soliden Tumoren und die dabei involvierten Signalwege zu bestimmen.

Methoden: Extrinsische Apoptose wurde in Krebszelllinien durch Behandlung sowohl mit einem Fas Agonist-Antikörper (CH11), als auch rekombinant exprimiertem TRAIL stimuliert, und der Einfluss einer RNAi-vermittelten Verringerung der MACC1 Expression auf den Verlauf der Apoptose analysiert. Die Viabilität der behandelten Zellen wurde mittels MTT bestimmt. Zelltod durch Apoptose wurde durch PARP- und Caspaseaktivität bestätigt und durch FACS-Analyse quantifiziert. Die während der Apoptose aktivierte Jak/STAT Signalkaskade wurde durch Expressionsanalyse und Phosphorylierungsrate ihrer Schlüsselfaktoren bestimmt und durch Behandlung der Zellen mit dem Jak1/2-spezifischen Inhibitor Ruxolitinib geprüft Die Abhängigkeit der Apoptoseinduktion von den Apoptose-regulierenden Faktoren Mcl-1 und Bax/Bak wurde in Zelllinien mit einer Überexpression (Mcl-1) oder Deletion (Bax/Bak) dieser Gene untersucht. Korrelationsanalysen der Expressionslevel von MACC1 und Mcl-1 in humanen Primärtumoren wurden in Expressionsdatensätzen aus zwei öffentlich verfügbaren Patientenkohorten (GEO Datenbank) durchgeführt.

Ergebnisse: Wir konnten zeigen, dass die Verringerung der MACC1-Expression in Krebszelllinien die Fas-vermittelte Apoptose verstärkt. Diese Studie belegt zum ersten Mal, dass der Jak/STAT-Signalweg durch den Expressionsgrad von MACC1 reguliert werden kann. Die Verringerung der MACC1-Expression reduzierte gleichzeitig aktivierende Phosphorylierungen in STAT1 (Y^{701}) und STAT3 (Y^{705}). Die Änderungen im Phosphorylierungsstatus der STAT-Proteine bedingte auch die nachfolgende Expressionsregulation der Apoptose-assoziierten Zielgene Mcl-1 und Fas. Die Inhibition des Jak/STAT-Signalweges durch Ruxolitinib zeigte vergleichbare Muster in der Änderung der betrachteten Gene und der Sensitivierung gegenüber Fas-vermittelter Apoptose. Die Sensitivierung der Krebszelllinien gegenüber Apoptoseinduktion durch verringerte Mcl-1-Expression hing dabei von den beiden pro-apoptotischen Faktoren Bax

und Bak ab. Die Verringerung der MACC1-Expression erhöhte auch die Sensitivität gegenüber TRAIL-induzierter Apoptose. Eine Überexpression von MACC1 erhöhte den Phosphorylierungsgrad von STAT1/3 und damit die Expression von Mcl-1. Dieser Effekt wurde durch Ruxolitinib inhibiert. Die hier beschriebene Expressionsregulation von MACC1 und Mcl-1 erhält dabei klinische Relevanz durch eine positive Expressionskorrelation in Tumorproben von Krebspatienten.

Schlußfolgerungen: In dieser Studie wurde ein bislang unbeschriebener Regulationsmechanimus der STAT1/3 - Mcl-1 - Bax/Bak - Signalkaskade identifiziert, bei dem eine Verringerung der MACC1-Expression Krebszelllinien gegenüber der Induktion extrinsischer Apoptose sensibilisiert. Eine Reduktion der MACC1-Expression in Krebspatienten, kombiniert mit der Induktion extrinsischer Apoptose, könnte eine neuartige Therapie zur Reduktion der Tumorprogression und –metastasierung darstellen.

Abstract

Introduction: Apoptosis is a vital mechanism which is deregulated during cancer progression. Metastasis associated in colon cancer 1 (MACC1) was identified as a novel player in cancer progression. However, the role of MACC1 in death receptor mediated apoptosis regulation remains to be elucidated. Thus, we studied the impact of MACC1 expression on death receptor mediated apoptosis and its regulatory mechanisms in solid cancers.

Methodology: Apoptosis induction in solid cancer cell lines was achieved using a Fas agonist antibody (CH11) or recombinant TRAIL treatment upon MACC1 knockdown. Cell viability analysis was performed by MTT assay; apoptosis was quantified by FACS and was confirmed by PARP and caspase activity analysis. Changes in signaling mechanism and key apoptotic protein expression was analyzed by Western blot. STAT signaling inhibition was achieved by Jak1/2 specific inhibitor (ruxolitinib) treatment. Mcl-1 overexpression and Bax/Bak knockout cell lines were used to study their importance in MACC1 signaling. Correlation analysis of MACC1 and Mcl-1 expression was performed in two publicly available (GEO database) cohorts of patient derived tumor specimens.

Results: We showed that MACC1 knockdown enhances Fas mediated apoptosis in cancer cells. For the first time, our study provides evidence for STAT signaling as a target of MACC1. MACC1 knockdown drastically reduced STAT1 (Y^{701}) and STAT3 (Y^{705}) activating phosphorylation, thereby regulating the expression of its apoptosis targets Mcl-1 and Fas. Ruxolitinib treatment mimicked MACC1 knockdown mediated molecular signatures and apoptosis sensitization to Fas activation. Despite the increased Fas expression, the reduced Mcl-1 expression was instrumental in apoptosis sensitization. This reduced Mcl-1 mediated apoptosis sensitization was both Bax and Bak dependent. Additionally, MACC1 knockdown also increased TRAIL induced apoptosis. MACC1 overexpression enhanced STAT1/3 phosphorylation and increased Mcl-1 expression, which was abrogated by ruxolitinib. The central role of Mcl-1 was strengthened by the resistance of Mcl-1 overexpressing cells to apoptosis induction upon MACC1 knockdown. Importantly, the clinical relevance of Mcl-1 regulation by MACC1 was evidenced by their positive expression correlation in patient derived tumor specimens.

Conclusions: Our study reveals a novel death receptor mediated apoptosis regulatory mechanism by MACC1 through modulated STAT1/3-Mcl-1-Bax/Bak axis. Therefore combining MACC1 inhibition with death receptor activation might be a novel therapeutic strategy to prevent solid cancer progression.

Affidavit

I, Harikrishnan Radhakrishnan certify under penalty of perjury by my own signature that I have submitted the thesis on the topic “MACC1 regulates death receptor mediated apoptosis in solid cancers through STAT1/3 – Mcl-1 signaling”. I wrote this thesis independently and without assistance from third parties, I used no other aids than the listed sources and resources.

All points based literally or in spirit on publications or presentations of other authors are, as such, in proper citations (see “uniform requirements for manuscripts (URM)” the ICMJE www.icmje.org) indicated. The section on methodology (in particular practical work, laboratory requirements, statistical processing) and results (in particular images, graphics and tables) corresponds to the URM (s.o) and are answered by me. My contribution in the selected publication for this dissertation corresponds to those that are specified in the following joint declaration with the responsible person and supervisor.

The importance of this affidavit and the criminal consequences of a false affidavit (section 156,161 of the Criminal Code) are known to me and I understand the rights and responsibilities stated therein.

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Date: Signature

Detailed Declaration of Contribution

Harikrishnan Radhakrishnan had the following share in the following publication:

Publication: **Harikrishnan Radhakrishnan**, Katharina Ilm, Wolfgang Walther, Senji Shirasawa, Takehiko Sasazuki, Peter T. Daniel, Bernhard Gillissen, Ulrike Stein. MACC1 regulates Fas mediated apoptosis through STAT1/3 – Mcl-1 signaling in solid cancers. Cancer Letters, 2017, 403: 231-245.
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Contribution in detail:

Based on the previous work on MACC1, Prof. Ulrike Stein conceived the project evaluating the role of MACC1 in apoptosis regulation in cancer cells. Harikrishnan Radhakrishnan in the capacity of a doctoral fellow identified the role of MACC1 in death receptor mediated apoptosis signaling. The project was independently developed by Harikrishnan Radhakrishnan with minimal supervision. Most importantly, all experiments corresponding to the above mentioned publication was conceived, designed and performed by Harikrishnan Radhakrishnan. This includes all wet lab works, data collection and analysis. Further, all statistical analyses and its interpretation were performed by Harikrishnan Radhakrishnan. Manuscript was prepared by Harikrishnan Radhakrishnan and was read and critically reviewed by Prof. Ulrike Stein. Harikrishnan Radhakrishnan handled the revision process following peer-review including additional necessary experiments and manuscript revision. For expert advice and experimental designs concerning apoptosis signaling, Harikrishnan Radhakrishnan initiated a collaboration with the lab of Prof. Peter T. Daniel who also later served as his PhD committee member. Cell lines overexpressing Mcl-1 and Bax/Bak knockouts were obtained from Dr. Bernhard Gillissen (AG Prof. Daniel) who also supported in the design and analysis of apoptosis assays using flow cytometry. Hkh-2 and Hke-3 cell lines were provided by Prof. Senji Shirasawa and Prof. Takehiko Sasazuki upon a Material transfer agreement for sharing authorship. Dr. Katharina Ilm provided ruxolitinib for STAT inhibition and supported its optimization in signaling studies. LS174T, PaTu8902 and T47D cells were obtained with the help of Prof. Wolfgang Walther, who also helped in reviewing the experimental design and manuscript preparation. The final version of the manuscript was read and approved by all co-authors prior to publication.

Signature, date and stamp of the supervising University teacher

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Signature of the doctoral candidate

Excerpt of the Journal Summary List (Web of Knowledge[SM])

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Journal Data Filtered By: Selected JCR Year: 2016; Selected Editions: SCIE; Selected Categories: 'ONCOLOGY'; Selected Category Scheme: WoS

Rank	Full Journal Title	JCR Abbreviated Title	ISSN	Journal Impact Factor	Eigenfactor Score
1	CA-A CANCER JOURNAL FOR CLINICIANS	CA-CANCER J CLIN	0007-9235	187.040	0.064590
2	NATURE REVIEWS CANCER	NAT REV CANCER	1474-175X	37.147	0.084950
3	LANCET ONCOLOGY	LANCET ONCOL	1470-2045	33.900	0.121930
4	CANCER CELL	CANCER CELL	1535-6108	27.407	0.102930
5	JOURNAL OF CLINICAL ONCOLOGY	J CLIN ONCOL	0732-183X	24.008	0.284800
6	Nature Reviews Clinical Oncology	NAT REV CLIN ONCOL	1759-4774	20.693	0.026770
7	Cancer Discovery	CANCER DISCOV	2159-8274	20.011	0.053450
8	JAMA Oncology	JAMA ONCOL	2374-2445	16.559	0.011300
9	JNCI-Journal of the National Cancer Institute	JNCI-J NATL CANCER I	0027-8874	12.589	0.062530
10	ANNALS OF ONCOLOGY	ANN ONCOL	0923-7534	11.855	0.090980
11	LEUKEMIA	LEUKEMIA	0887-6924	11.702	0.059800
12	CLINICAL CANCER RESEARCH	CLIN CANCER RES	1078-0432	9.619	0.140990
13	BIOCHIMICA ET BIOPHYSICA ACTA-REVIEWS ON CANCER	BBA-REV CANCER	0304-419X	9.452	0.009460
14	SEMINARS IN CANCER BIOLOGY	SEMIN CANCER BIOL	1044-579X	9.141	0.012370
15	CANCER RESEARCH	CANCER RES	0008-5472	9.122	0.149180
16	CANCER TREATMENT REVIEWS	CANCER TREAT REV	0305-7372	8.589	0.014420
17	Cancer Immunology Research	CANCER IMMUNOL RES	2326-6066	8.284	0.013910
18	Liver Cancer	LIVER CANCER	2235-1795	7.854	0.001550
19	NEURO-ONCOLOGY	NEURO-ONCOLOGY	1522-8517	7.786	0.024280
20	OncoImmunology	ONCOIMMUNOLOGY	2162-402X	7.719	0.015390
21	ONCOGENE	ONCOGENE	0950-9232	7.519	0.080060
22	JOURNAL OF PATHOLOGY	J PATHOL	0022-3417	6.894	0.026950
23	Journal of Thoracic Oncology	J THORAC ONCOL	1556-0864	6.595	0.032240
24	INTERNATIONAL JOURNAL OF CANCER	INT J CANCER	0020-7136	6.513	0.073930
25	CANCER LETTERS	CANCER LETT	0304-3835	6.375	0.040280
26	Journal of Hematology & Oncology	J HEMATOL ONCOL	1756-8722	6.350	0.007920
27	BREAST CANCER RESEARCH	BREAST CANCER RES	1465-542X	6.345	0.023850
28	Therapeutic Advances in Medical Oncology	THER ADV MED ONCOL	1758-8340	6.294	0.002420
29	Advances in Cancer Research	ADV CANCER RES	0065-230X	6.267	0.003210
30	SEMINARS IN ONCOLOGY	SEMIN ONCOL	0093-7754	6.212	0.008120
31	Molecular Cancer	MOL CANCER	1476-4598	6.204	0.018240
32	BRITISH JOURNAL OF CANCER	BRIT J CANCER	0007-0920	6.176	0.073220
33	Blood Cancer Journal	BLOOD CANCER J	2044-5385	6.126	0.006320
34	EUROPEAN JOURNAL OF CANCER	EUR J CANCER	0959-8049	6.029	0.048010
35	CANCER	CANCER-AM CANCER SOC	0008-543X	5.997	0.074110

Journal : Cancer Letters **Impact Factor : 6.375**

Category : Oncology **Eigenfactor Score : 0.040280**

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Selected Publication

Printed copy of the original publication

Harikrishnan Radhakrishnan, Katharina Ilm, Wolfgang Walther, Senji Shirasawa, Takehiko Sasazuki, Peter T. Daniel, Bernhard Gillissen, Ulrike Stein. MACC1 regulates Fas mediated apoptosis through STAT1/3 – Mcl-1 signaling in solid cancers. Cancer Letters, 2017, 403: 231-245.

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Curriculum Vitae

Mein Lebenslauf wird aus datenschutzrechtlichen Gründen in der elektronischen Version meiner Arbeit nicht veröffentlicht.

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Mein Lebenslauf wird aus datenschutzrechtlichen Gründen in der elektronischen Version meiner Arbeit nicht veröffentlicht.

List of Publications

Journal articles:

1. **Harikrishnan Radhakrishnan**, Katharina Ilm, Wolfgang Walther, Senji Shirasawa, Takehiko Sasazuki, Peter T Daniel, Bernhard Gillissen, Ulrike Stein. 2017. MACC1 regulates Fas mediated apoptosis through STAT1/3 – Mcl-1 signaling in solid cancers. Cancer Letters, 403: 231-245. https://doi.org/10.1016/j.canlet.2017.06.020

2. Indumathi Somasundaram, Rashmi Mishra, **Harikrishnan Radhakrishnan**, Rajkumar Sankaran, Venkata Naga Srikanth Garikipati, Dhanasekaran Marappagounder. 2015. Human adult stem cells maintain a constant phenotype profile irrespective of their origin, basal media, and long term cultures. Stem cell International, 146051. http://dx.doi.org/10.1155/2015/146051

3. Indumathi Somasundaram, **Harikrishnan Radhakrishnan**, Rajkumar Sankaran, Dhanasekaran Marappagounder. 2015. Immunophenotypic comparison of heterogenous non-sorted versus sorted mononuclear cells from human umbilical cord blood: a novel cell enrichment approach. Cytotechnology, 67(1): 107-114. https://dx.doi.org/10.1007%2Fs10616-013-9663-2

4. Indumathi Somasundaram, Rashmi Mishra, **Harikrishnan Radhakrishnan**, Rajkumar Sankaran, Neha Kantawala, Dhanasekaran Marappagounder. 2014. Lineage depletion of stromal vascular fractions isolated from human adipose tissue: a novel approach towards cell enrichment technology. Cytotechnology, 66(2): 219-228. https://dx.doi.org/10.1007%2Fs10616-013-9556-4

5. Dhanasekaran Marappagounder, Indumathi Somasundaram, **Harikrishnan Radhakrishnan**, Rashmi Mishra, Lissa Rachel Philip, Rajkumar Sankaran, Sudarsanam Dorairaj. 2013. Human omentum fat-derived mesenchymal stem cells transdifferentiates into pancreatic islet-like cluster. Cell Biochemistry and Function, 31(7): 612-619. https://doi.org/10.1002/cbf.2948

6. Indumathi Somasundaram, **Harikrishnan Radhakrishnan**, Rashmi Mishra, Rajkumar Sankaran, Padmapriya V, Lissa Rachel Philip, Dhanasekaran

Marappagounder. 2013. Comparison of feto-maternal organ derived stem cells in facets of immunophenotype, proliferation and differentiation. Tissue and Cell, 45(6): 434-442. https://doi.org/10.1016/j.tice.2013.07.007

7. Indumathi Somasundaram, **Harikrishnan Radhakrishnan**, Rajkumar Sankaran, Sudarsanam Dorairaj, Dhanasekaran Marappagounder. 2013. Prospective biomarkers of stem cells of human endometrium and fallopian tube compared with bone marrow. Cell and Tissue Research, 352(3): 537-549. https://doi.org/10.1007/s00441-013-1582-1

8. Dhanasekaran Marappagounder, Indumathi Somasundaram, Lissa Rachel Philip, **Harikrishnan Radhakrishnan**, Rajkumar Sankaran, Sudarsanam Dorairaj. 2013. A comprehensive study on optimization of proliferation and differentiation potency of bone marrow derived mesenchymal stem cells under prolonged culture condition. Cytotechnology, 65(2): 187-197. https://dx.doi.org/10.1007%2Fs10616-012-9471-0

Book chapters:

1. Indumathi Somasundaram, Rashmi Mishra, **Harikrishnan Radhakrishnan**, Dhanasekaran Marappagounder. 2015. Subcutaneous adipose tissue-derived stem cells: advancement and applications in regenerative medicine. Regenerative Medicine (eds. Niranjan Bhattacharya, Phillip George Stubblefield). pp 91-112. DOI: 10.1007/978-1-4471-6542-2_10

2. Indumathi Somasundaram, **Harikrishnan Radhakrishnan**, Dhanasekaran Marappagounder. 2015. Redundant human omentum fat: a leap towards regenerative medicine. regenerative medicine (eds. Niranjan Bhattacharya, Phillip George Stubblefield). pp 125-133. DOI: 10.1007/978-1-4471-6542-2_12

3. Indumathi Somasundaram, **Harikrishnan Radhakrishnan**, Rashmi Mishra, Rajkumar Sankaran, Dhanasekaran Marappagounder. 2014. Surface antigenic profiles of stem cells from the human bone marrow, subcutaneous fat, and omentum fat. Stem cells in Aesthetic Procedures (eds. Melvin A. Shiffman, Alberto Di Giuseppe, Franco Bassetto). pp 41-66. DOI: 10.1007/978-3-642-45207-9_4

Oral presentations:

June 2017 : “MACC1 regulates Fas mediated apoptosis in solid cancers through STAT1/3 - Mcl-1 signaling” at the 11th International PhD student cancer conference (IPSCC) held at Berlin, Germany

May 2016 : “MACC1 knockdown sensitizes colorectal cancer cells to FasL/FasR mediated apoptosis through modulation of STAT - Mcl-1 signaling axis” at an international conference on “Changing views in cancer” held at Berlin, Germany

Jun 2015 : “MACC1 interacts with FasL endowing colorectal cancer cells a migratory advantage” at the Young scientists in cancer research, a Satellite symposium of the international conference on “Making walls history: Overcoming treatment barriers in cancer” held at Berlin, Germany

Oct 2011 : “In vivo Drug Factory“, representing team VIT_Vellore at the Regional Jamboree: Asia for International Genetically Engineered Machine Competition, iGEM – Hong Kong

Oct 2010 : “Nano-liposomes as drug delivery agents”, “Blue print- Chemical engineering” at technical symposium- Tathva-’10 of National Institute of Technology, Calicut (NIT-C), India

Poster presentations:

Sept 2016 : “MACC1 modulates STAT – Mcl-1 signaling axis and sensitizes colorectal cancer cells to FasR mediated apoptosis”. At The 7th EMBO meeting in Mannheim, Germany

May 2016 : “MACC1 knockdown sensitizes colorectal cancer cells to FasL/FasR mediated apoptosis through modulation of STAT - Mcl-1 signaling axis”. At an international conference on “Changing views in cancer” held at Berlin, Germany

Jun 2015 : “MACC1 interacts with FasL endowing colorectal cancer cells a migratory advantage”. At an international conference on “Making walls history: Overcoming treatment barriers in cancer” held at Berlin, Germany

May 2014 : “MACC1 links metastasis and chemoresistance in colorectal cancer”. At an International conference on “From Omics to Novel Therapies in Cancer” held at Berlin, Germany

May 2014 : “MACC1 impart chemoresistant phenotype in colorectal cancer”. At MDC/FMP Campus Symposium “Your Bench and Beyond” held at Berlin, Germany

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